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Breast Cancer, Tamoxifen & Beyond: Estrogen and Estrogen Receptors

Acknowledgments

Breast Cancer, Tamoxifen & Beyond: Estrogen and Estrogen Receptors

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THE ENDOCRINE SOCIETY®

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COVER IMAGE: Each year, hundreds of thousands of women in the United States are diagnosed with breast cancer. Because of decades of research into the basic biology of cancer and the hormone estrogen, major improvements have been made in survival rates, prevention, and quality of life related to breast cancer. From endocrinologists and biochemists studying the molecular structure of estrogen and its receptor to physiologists and cancer researchers seeking to understand breast cancer at the cellular level, fundamental research has played an invaluable role in developing breakthrough therapies like tamoxifen. *MCF-7 cells image courtesy of Berkeley Imaging Center and Dr. Gary Firestone; Estrogen-ER image courtesy of Dr. David S. Goodsell, Scripps Research Institute; all other images (c) Getty Images, used with permission.*

Breast Cancer, Tamoxifen & Beyond: Estrogen and Estrogen Receptors

"Research does not travel in straight lines and observations in one field of science often become major discoveries in another." Lerner and Jordan 1990 *Cancer Research* 50:4177.

Hormones control our lives. These chemical messengers circulate through the blood from one organ or tissue to another, controlling and coordinating a wide range of bodily functions, including growth, development, metabolism, and reproduction. Hormones and the tissues that produce them make up the endocrine system, which exerts its control over us through molecular interactions between hormones and cells.

Throughout a woman's life, her cells and sex hormones interact in different ways in the ovaries, uterus, breasts, and other tissues. The details of these interactions—the type of cells or hormones, the location and timing of the interaction, the responsiveness of the cells—determine the outcome. As she enters her reproductive years, the interaction between cells and sex hormones produces puberty. Her body goes through monthly changes orchestrated by the cells and hormones of her reproductive system, until her reproductive years end in menopause. Other interactions allow a woman's body to sustain a pregnancy, give birth to a child, and nourish that child with milk.

Sex hormones also appear to play a role in breast cancer. More than 200,000 American women are diagnosed with breast cancer each year, and 40,000 American women die each year from breast cancer. It is the most common cause of death in women aged 45–55. Cancer is an extremely complicated disease. In fact, "cancer" is a catch-all term that includes many different diseases affecting different cells. The causes of cancer are just as complicated. Factors that affect the development of breast cancer include heredity, environmental factors (such as exposure to radiation and certain chemicals), and lifestyle factors (such as obesity and alcohol use). A woman's reproductive history may also have a profound effect on her breast cancer risk.

The connection between a woman's hormonal status and her risk of developing breast cancer has been suspected since Bernardino Ramazzini noted in 1713 that nuns were more likely to develop breast cancer than women who had borne children. Only in the last century has that connection been studied scientifically and used to treat cancer.

Since it was introduced in the 1970s, the breast cancer drug tamoxifen has prolonged the lives of millions of women by changing the way cells and hormones interact. At the time it was developed, however, little was known about how the drug worked. By studying how cells and hormones work together in healthy and diseased tissues, scientists are gaining new insights about drugs like tamoxifen. This new understanding is resulting in new approaches to drug design and in the application of this knowledge to address a variety of women's (and men's) health issues.

"Ovarian Irritation" & "Internal Secretions"

Near the end of the 19th century, George Beatson, a Scottish surgeon, observed that the egg-producing organs of sheep (Figure 1), the ovaries, seemed to affect milk production. Beatson suspected that "ovarian irritation" might be a factor in breast cancer, so he removed the ovaries of a woman with advanced breast cancer. She survived for nearly four years, a remarkable success in those days. Beatson and others continued to treat breast cancer by removing the ovaries, and they were successful about a third of the time.

Leo Loeb performed some of the earliest laboratory studies on breast (mammary) cancer at the University of Pennsylvania. Loeb obtained some mice that were prone to developing mammary cancer and removed their ovaries, dramatically reducing the cancer

Figure 1: Sheep and chickens prove important in breast cancer research. Ewes and hens served as crucial animal models in early studies to understand how estrogen worked and how it affected breast tumor development. Animal models often play an invaluable role on the path of discovery towards understanding and treating disease. *Photo courtesy of the Turtleback Farm Inn.*

rate. In 1916, he proposed that some "internal secretion" affected the growth of cancer cells. What that secretion was, and how it worked, would take decades to work out.

Discovery of Estrogen

In 1922, Edward Doisy and Edgar Allen were close friends and colleagues at Washington University in St. Louis. One day, while they were traveling home together in Doisy's Model T, Allen asked Doisy for his help. Allen was studying the estrous cycle of mice. Similar to the human menstrual cycle, the estrous cycle produces changes in the mouse reproductive tract associated with estrus or "heat", the ability to become pregnant. Although he was working intensively on other projects, Doisy agreed to try to isolate some of the active compounds from ovaries.

To study something you have to be able to measure it, and Allen and Doisy developed a test to measure the then unknown substance responsible for what they called "estrogenic activity." When an estrogenic substance was injected into mice whose ovaries had been removed, it caused specific vaginal changes associated with estrus. With the Allen-Doisy bioassay in hand, Doisy attempted to isolate estrogenic compounds from pig ovaries he had obtained from slaughterhouses. Progress was slow.

Doisy's work was helped along in 1927, when Selmar Ascheim and Bernhard Zondek found estrogenic activity in the urine of pregnant women. Ascheim and Zondek went on to develop the first reliable pregnancy test, in which urine from pregnant women could induce specific changes in a mouse's ovaries. This evolved into the "rabbit test" which detected those changes in rabbits. These tests (and modern pregnancy tests) measured human chorionic gonadotropin (hCG), a hormone present only in pregnant women. It was not the "internal secretion" sought by Doisy.

Nevertheless, Doisy credited Ascheim and Zondek with making his life easier, since he did not need to obtain pig ovaries from slaughterhouses, but could just collect urine from pregnant women. He later recalled, "…my pregnant friends were enlisted and amusedly they responded; perhaps the best contributor was a niece of my wife, Alice." As his studies progressed, they required more and more urine—gallons of it. He turned to Miss Terry, the nurse in a local obstetrics clinic, for help. Doisy told the story of how one of his colleagues had been transporting large bottles of urine to the laboratory in his car when he was stopped by a police officer. This being prohibition, the officer suspected that the fluid in the back seat might be illegal bootleg liquor. One whiff of the fluid told the officer that the scientist was no bootlegger.

By 1929, Doisy had isolated the first estrogen from urine. At about the same time, two other groups of scientists, led by Adolf Butenandt in Germany and Guy Marrian in the UK (Figure 2), independently

Figure 2: The 'estrogen musketeers,' as they were once known, Edward Doisy (left) and Adolf Butenandt (right). Together with Guy Marrian (the 'third musketeer', not shown), these researchers were the first to successfully isolate estrogen from urine, and all three were eventually awarded Nobel prizes. *Photo © The Nobel Foundation.*

isolated estrogens from urine. In 1936, Doisy finally succeeded in isolating estradiol (the most potent estrogenic compound) from 4,000 kg of pig ovaries.

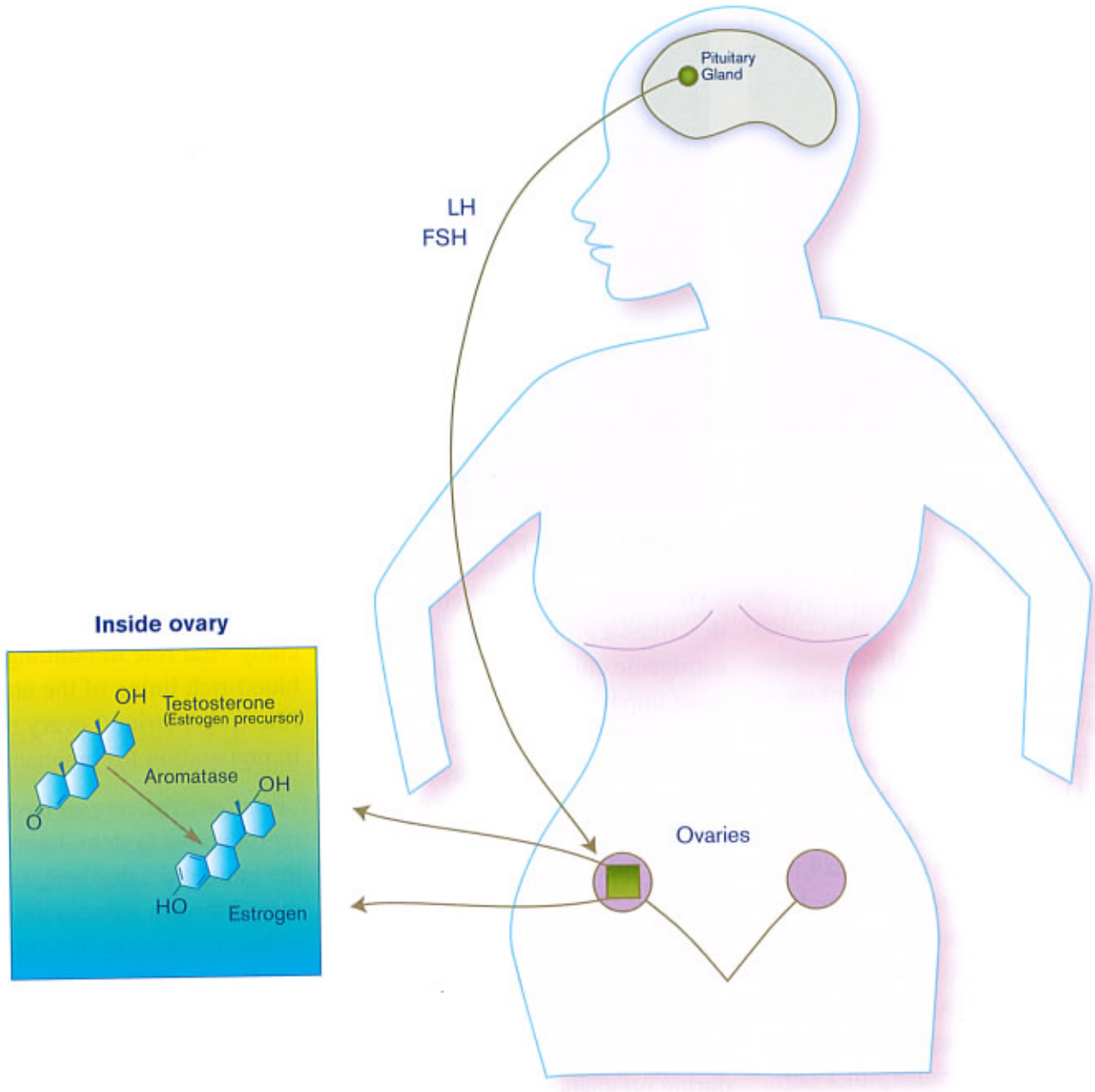
Butenendt and Marrian went on to analyze the structural relationships between estrogen and other related hormones (together called steroid hormones) and found that they were related to cholesterol. (See the *Breakthroughs in Biosciences* article, "Cholesterol: From Biochemical Riddle to Blockbuster Day for Heart Disease"). Butenandt shared the Nobel Prize in Chemistry in 1939 with Leopold Ruzicka for his work with sex hormones. Because of the political climate in Europe, Butenandt did not receive his medal until 1949. Doisy won the 1943 Nobel Prize in Medicine, but for his later work on vitamin K and not for his work with estrogen.

What Does Estrogen Do?

The word "estrogen" actually describes a group of interrelated estrogenic compounds. The three most important human estrogens are estradiol, estrone, and estriol. Estradiol is the most thoroughly studied and most active of the estrogens, and is the major estrogen in premenopausal women. Estrone is weaker than estradiol and is the major estrogen in postmenopausal women. Estriol is the weakest of the three and is produced in large quantities during pregnancy. The body can convert each of these three estrogens into any of the others. There are more than fifty less abundant and less studied forms of estrogen in the body; some are breakdown products of the three major estrogens, and many have biological activity.

Estrogen is necessary for fertility, for the growth and maturation of the sex organs, and for the development of secondary sex characteristics (such as breasts and pubic hair). Estrogen is one of the hormones that regulate the menstrual cycle, playing an especially vital role in building up the blood-rich lining of the uterus (the endometrium) every month in preparation for pregnancy.

Estrogen's effects go beyond the reproductive system. Estrogen in the brain regulates body temperature. Estrogen reduces the risk of heart disease by limiting the production of LDL (low-density lipoproteins; the "bad cholesterol") and promoting the production of HDL (high-density lipoproteins; the "good cholesterol"). Estrogen also maintains bone density and strength. These other functions of estrogen become apparent at menopause, when estrogen levels fall, producing hot flashes, increased risk of heart disease, and osteoporosis (see *Breakthroughs in Biosciences* article, "Bone Builders: The Discoveries Behind Preventing & Treating Osteoporosis"). Menopausal symptoms can be



Sidebar 1:

Estrogen synthesis: How, where, and when is estrogen made?

Estrogen's resemblance to cholesterol is no coincidence. Estrogen is made from cholesterol through a series of chemical modifications guided by proteins called enzymes. The last step of estrogen synthesis requires an enzyme called aromatase. Aromatase changes testosterone (a steroid sex hormone usually associated with men, but present in small amounts in women) to estradiol, the most potent of the estrogens.

In premenopausal women, estrogen is mainly produced in the ovaries, in response to a signal (LH, or luteinizing hormone) from the pituitary gland at the base of the brain. As a girl reaches puberty, her estrogen levels rise. Estrogen levels fluctuate throughout the menstrual cycle, and are at their lowest levels just before the menstrual flow starts. During pregnancy, the placenta contributes to high levels of estrogen. After menopause, women produce very little estrogen.

There are sites in the body besides the ovaries that produce estrogen. The adrenal glands (just over the kidneys) produce estrogen precursors that can be converted to estrogen by aromatase present in the adrenal glands, breasts, and other tissues. After menopause, although the ovaries no longer produce estrogen, estrogen can be produced locally in the breast. Men can produce moderate levels of estrogen in the testes throughout their lives.

eased by hormone replacement therapy, but questions remain about its safety, notably in its relationship to cancer.

Estrogen and Breast Cancer

Once estrogen had been isolated in the laboratory, researchers began to study the effects of large amounts of estrogen on experimental animals. Like Leo Loeb, Antoine Lacassagne (Figure 3) used cancer-prone strains of mice to show that hereditary factors play a large part in determining the risk of developing mammary cancer. Loeb had removed ovaries to reduce the risk of cancer. In other studies, Lacassagne injected mice repeatedly with estrogen and increased the likelihood that they would develop mammary cancer. In 1936, he predicted that breast cancer might be treated or prevented by blocking the action of estrogen. As we shall see, his prediction would prove to be prophetic.

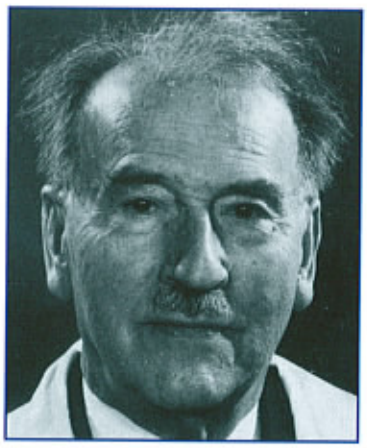


Figure 3: Antoine Lacassagne (1884-1971): French researcher Antoine Lacassgne performed some of the earliest experiments linking estrogen to breast cancer in mice. He predicted in 1936 that interfering with the actions of estrogen might prove useful in treating or preventing breast cancer. *Photo courtesy of Musee / Institut Pasteur.*

But what is cancer? Lacassagne described it as "…the illimitable power of multiplication in the organism, which is acquired by one cell and transmitted to its descendants. In this sense it would be a type of mutation, in the nature of an irreversible cytological change." He was right again. We now know that cancer is caused by damage to the genetic material in the nucleus of the cell; multiple mutations that result in uncontrolled growth and multiplication.

Estrogen's role in breast cancer is related to its role in normal, healthy breast tissue. Normal breast cells require estrogen to grow. Throughout her childbearing years, a woman's breasts (and the lining of her uterus) undergo repeated cycles of proliferation and death. If a mutated, cancerous breast cell also requires estrogen to grow, it will multiply in the presence of estrogen. As the cancerous cells continue to grow and multiply, they eventually produce a tumor. Cells from that tumor may spread, or metastasize, to other parts of the body.

Charles Huggins (Figure 4), a urologist at the University of Chicago in the 1950s and 1960s, treated rats and mice with cancer-causing chemicals and noted that some mammary tumors required sex hormones to grow. If the tumors were deprived of that hormone, they would often shrink. Huggins won the 1966 Nobel Prize in Physiology or Medicine for his work on sex hormones and cancer. In his Nobel lecture, Huggins acknowledged the importance of the interactions between cells and hormones when he said, "The quality of hormone-dependence resides in the tumor cells whereas their growth is determined by the host's endocrine status."

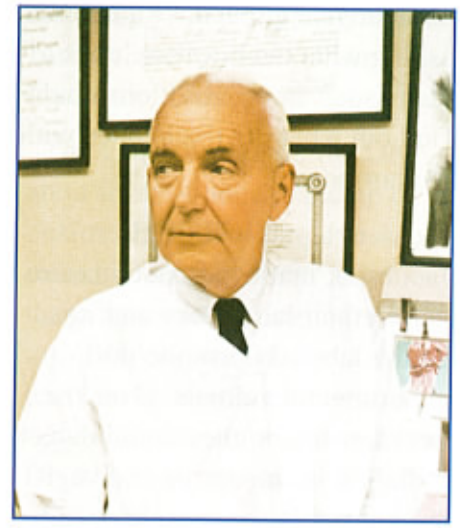


Figure 4: Charles Huggins (1901-1997): A urologist, Huggins won the 1966 Nobel Prize in Physiology or Medicine for his work showing the relationship between sex hormones and cancer. Above his desk at the University of Chicago, where he worked as a researcher, hung a plaque which proudly stated, "Discovery is our business." *Photo courtesy of the William P. Didusch Center for Urologic History.*

How Does Estrogen Work?

The reigning hypothesis in the early 1960s was that estrogen enhanced cell growth by increasing the overall enzymatic activity of the cell, but that didn't explain all of the experimental data. Researchers were stymied. Elwood Jensen, a chemist at the Ben May Laboratory for Cancer Research in Chicago (established by Charles Huggins), tried an

alternative approach. "Rather than asking what the hormone does to the tissue," he recalls, "one could find out what the tissue does with the hormone."

Jensen, along with Herbert Jacobson, made radioactive estrogen in their laboratory and injected the labeled hormone into experimental animals. Over the next few hours, they could detect radiation in the uterus and vagina, but not in the muscle or kidney. The tissues in which estrogen had biological activity were the same tissues to which the labeled hormone bound—the target tissues. This was the first time anyone had demonstrated tissue specificity for a hormone.

But what did it bind to? In 1966, Jack Gorski at the University of Illinois took the experiment one step further and broke apart the labeled tissues. After separating the components of the cells, he found the labeled hormone bound to a protein in the nucleus. That protein was the estrogen receptor (ER).

In the 1970s, Jensen developed a test to detect the ER in tumor cells. He proposed that cancer cells be analyzed for presence of the ER to see which ones were likely to respond to hormonal therapy. Approximately 70–80% of tumors from postmenopausal women have the ER, and 30–50% of tumors from premenopausal women are ER-positive. This probably explains why, 70 years earlier, about a third of premenopausal women with advanced breast cancer had responded favorably to ovary removal.

Fancy Mice and Test Tube Tumors

Since George Beatson's studies on the effect of ovary removal on advanced breast cancer, scientists have searched for ways to study breast cancer in the laboratory. At about the same time that Beatson was performing surgery in Scotland, a retired schoolteacher named Abbie Lathrop (Figure 5) began breeding "fancy" mice as pets in Massachusetts. Together with Leo Loeb, she demonstrated that some of her mouse strains had a high frequency of mammary tumors and others hardly ever developed cancer. Lathrop and Loeb published ten papers together. Scientists in a variety of disciplines began purchasing and studying her mice, and her colony grew to include over 10,000 mice. Today, nearly all of the mice used in research are descended from Abbie Lathrop's "fancy" mice.

Figure 5: Abbie Lathrop (1868-1918): A schoolteacher from Granby, MA, Abbie Lathrop began a collaboration with cancer researcher Leo Loeb, when she discovered that some of the "fancy" mice she was breeding for sale as pets had a high incidence of tumors. The raising of "fancy" mice, so called because they were bred for particular coat or eye colors or other beauty characteristics, was very popular in Victorian England and turn of the century America. Many of these inbred strains of mice would become important research animals for scientists, because their genetic uniformity made them powerful models for understanding basic biology and disease. *Redrawn from the Springfield Sunday Republican, October 5, 1913.*

Experiments in animals have yielded valuable insights into estrogen and breast cancer. Cancer cells taken from human tumors have also been invaluable in this research, but studying the disease in human cells proved difficult. Although these cells could grow for a while in laboratory dishes, they eventually died. In 1973, Herbert Soule, Samuel Brooks, and their colleagues, whose research was supported by the United States Public Health Service and the National Cancer Institute, reported that they had developed a new breast cancer cell line. This cell line, called MCF-7, came from a woman with advanced breast cancer. (Figure 6) The cell line could grow indefinitely in laboratory dishes and, even better, it possessed the ER. Today, MCF-7 cells are still the "workhorses" of ER research. They can be studied in laboratory dishes or implanted into mice with defective immune systems, essentially producing human tumors in experimental animals.

From Chickens to Coregulators

When Bert O'Malley joined the National Cancer Institute of the National Institutes of Health (NIH) in 1965, he expected to work on hormone chemistry for about a year. He did not suspect that it was to become his life's work. He later recalled discussions at coffee breaks about Jensen's studies of tissue binding with labeled estrogen. There was much controversy at the time as to whether Jensen's protein was a true receptor and how estrogen did what it did.

O'Malley began his research at a time when the field of molecular genetics was exploding. Scientists were finally beginning to answer the questions "How does a cell know what kind of cell it is?," "How does a cell make proteins?," and "How does it know which proteins to make?" Since Watson and Crick made their seminal discovery of the structure of DNA in 1953, the answers to those questions were beginning to appear. DNA, or deoxyribonucleic acid, is the stuff that makes up our genes. Virtually every cell in the body contains this "blueprint for life," a full set of instructions for every protein the body makes. But each cell makes only a fraction of those proteins.

The genes present in the DNA are a form of code made up of chains of four building blocks, called bases. The order in which these bases are strung together into DNA determines what protein that gene will represent or "encode." The information in the DNA is first "transcribed", or converted into a molecule called mRNA (messenger ribonucleic acid). The mRNA then brings that information to the cell's protein factories, the ribosomes, where the sequence of bases is "translated" into a sequence of amino acids, the building blocks of protein. It is the sequence of amino acids that makes each protein unique. But the question remained "How does a cell know which proteins to make?"

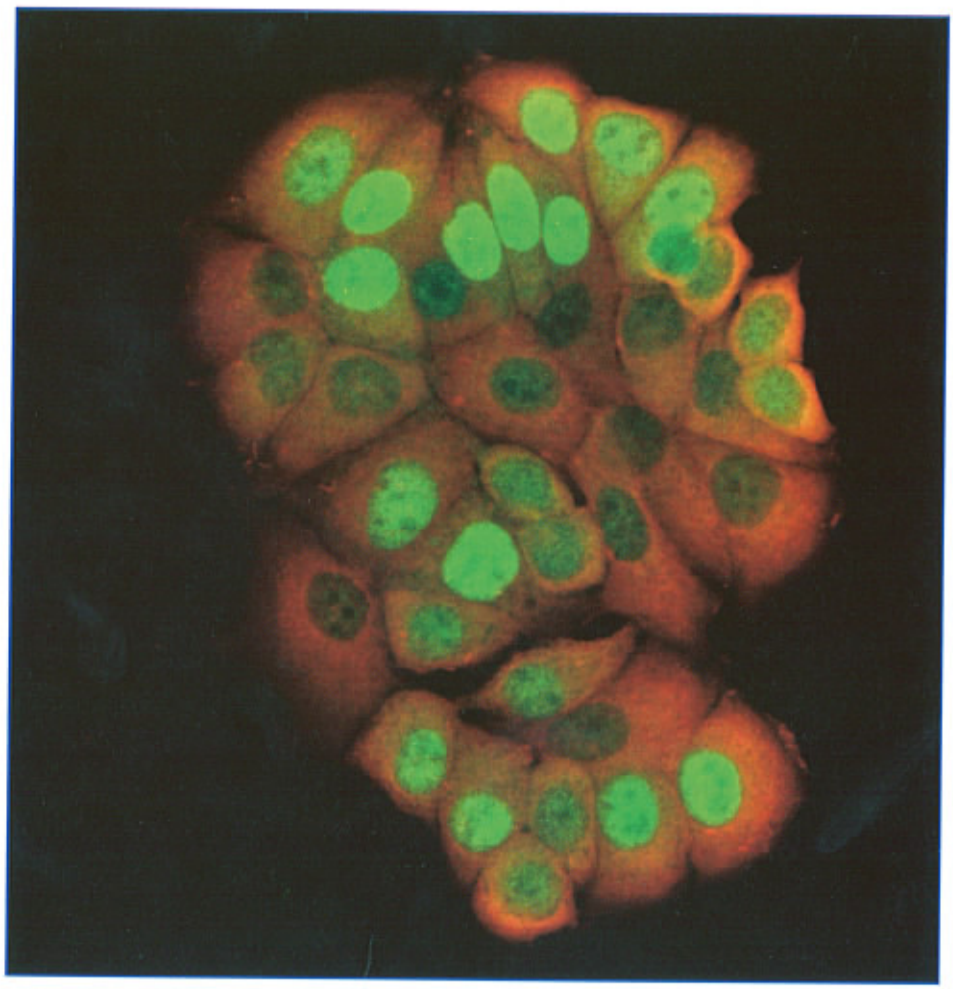


Figure 6: MCF-7 cells (shown here labeled with a fluorescent dye) are from the first human breast cancer cell line ever produced, and are still in use as a cellular model for studying the relationship between the estrogen receptor and breast cancer. *Image courtesy of Berkeley Imaging Center and Dr. Gary Firestone.*

At NIH, O'Malley developed a model to study this question. When young chickens are injected with estrogen, their oviducts (the tubes through which eggs pass) go through changes associated with sexual maturation. O'Malley and his mentor, Stan Korenman, suspected that certain proteins, such as ovalbumin (the major protein in egg whites) would also be produced. O'Malley showed that estrogen caused specific synthesis of new ovalbumin, an estrogen-associated protein, and not just a general increase in protein production. As his studies continued at Vanderbilt University, and later at Baylor College of Medicine, he

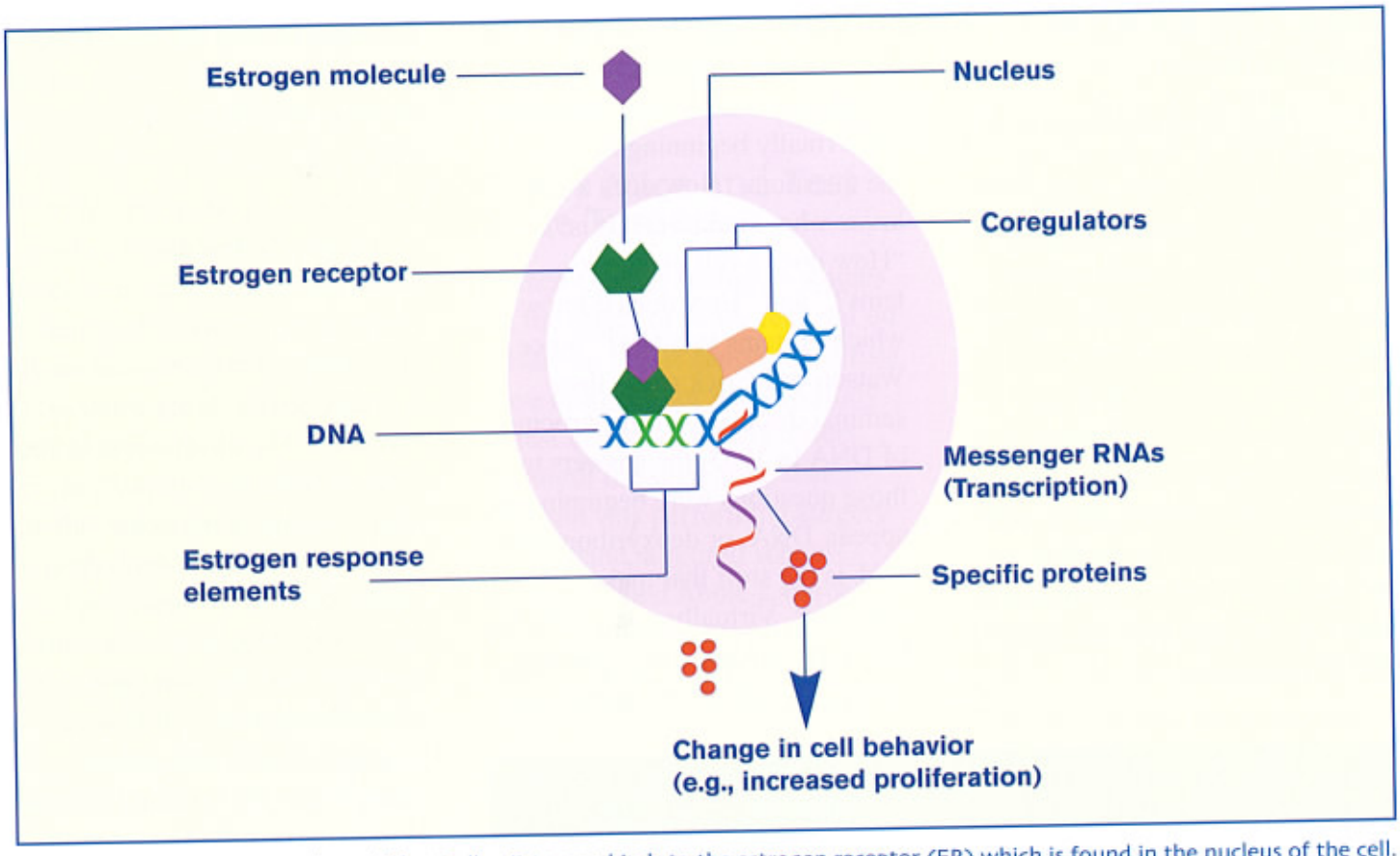


Figure 7: How Estrogen Works to Affect Cells - Estrogen binds to the estrogen receptor (ER) which is found in the nucleus of the cell. The estrogen-ER complex, including coregulators (additional proteins that also bind to the ER and promote or prevent further action), then binds to a specific spot on the cell's DNA, called the estrogen response element. This binding triggers transcription into messenger RNA (mRNA), which in turn is translated by the cell's internal processes into proteins. These proteins then interact with the body's cells, tissues, and organs to produce a physiological effect, such as increased cell growth or proliferation. **Please note:** For illustrative purposes, dimerization (pairing) of the estrogen receptor, as described in the text, is not shown. *Designed by Corporate Press, adapted from the National Cancer Institute, National Institutes of Health.*

showed that estrogen also caused new synthesis of ovalbumin-specific mRNA (as opposed to a general increase in mRNA), and O'Malley and others began to show how.

By the early 1990s, a model for ER action had begun to evolve. When estrogen binds to the ER in a cell, the estrogen-bound receptor pairs up with another estrogen-bound receptor and becomes activated. The activated estrogen-receptor complex can then bind directly to a region on the cell's DNA, the estrogen response element. This stimulates the transcription of an estrogen-specific gene into its corresponding mRNA, which can then be translated into the estrogen-specific protein. (Figure 7) In O'Malley's chick oviduct model, estrogen causes the cell to make ovalbumin, but in other cells, estrogen can induce the production of entirely different proteins. Because estrogen controls protein production by controlling transcription, this process is called transcriptional regulation. Understanding how estrogen regulates transcription was key to understanding not only how estrogen works, but how all cells work.

When O'Malley tried to reproduce estrogen-specific transcription in a laboratory dish, it didn't work. He noted that "there was some powerful magical protein component in nuclear extracts that greatly enhanced the action of the receptor." After another five years of work he isolated one of these "magical" components—a coregulator. Coregulators are proteins in the cell that bind to the estrogen-receptor complex, either promoting or preventing transcription. There are more than fifty proteins now known to bind to the ER; some stimulate transcription and some inhibit it.

The Receptor Takes Shape

Soon, the sequence of bases for the gene that encodes the protein of the ER was determined. This allowed chemists to predict how the amino acids would be put together and gave them some idea of the ER's shape. Other scientists used X-rays to produce an image of the ER, providing an even better idea of its shape.

By understanding how a protein is shaped, scientists can get an idea of how it works and how it interacts with other molecules. When estrogen binds to the ER, the shape of the receptor changes in subtle ways. These subtle changes can have profound functional effects, because they can change the way the activated receptor binds (or doesn't bind) to other molecules, such as coregulators. Coregulator binding (Figure 8), in turn, determines if the ER will promote transcription.

"Anti-Estrogens"

Between 1955 and 1980, surgery and chemotherapy were the treatments of choice for breast cancer. Chemotherapy targets rapidly dividing cells and, because it is not specific to cell type, it can have very toxic side effects. Hair follicles, the lining of the intestine, and the immune system all contain rapidly dividing cells, so chemotherapy drugs also cause hair loss, gastrointestinal distress, and immune suppression. Drugs that target breast cancer cells more specifically would produce fewer, less severe side effects. Progress toward this goal would come not from cancer researchers, but from laboratories looking for new methods of birth control.

Figure 9: Dora Richardson - A chemist at ICI Pharmaceuticals in England, Dora Richardson first synthesized a compound known then as ICI 46,474 and later called tamoxifen. This photo was taken on the occasion of her retirement in 1979. *Photo courtesy of Dr. V. Craig Jordan.*

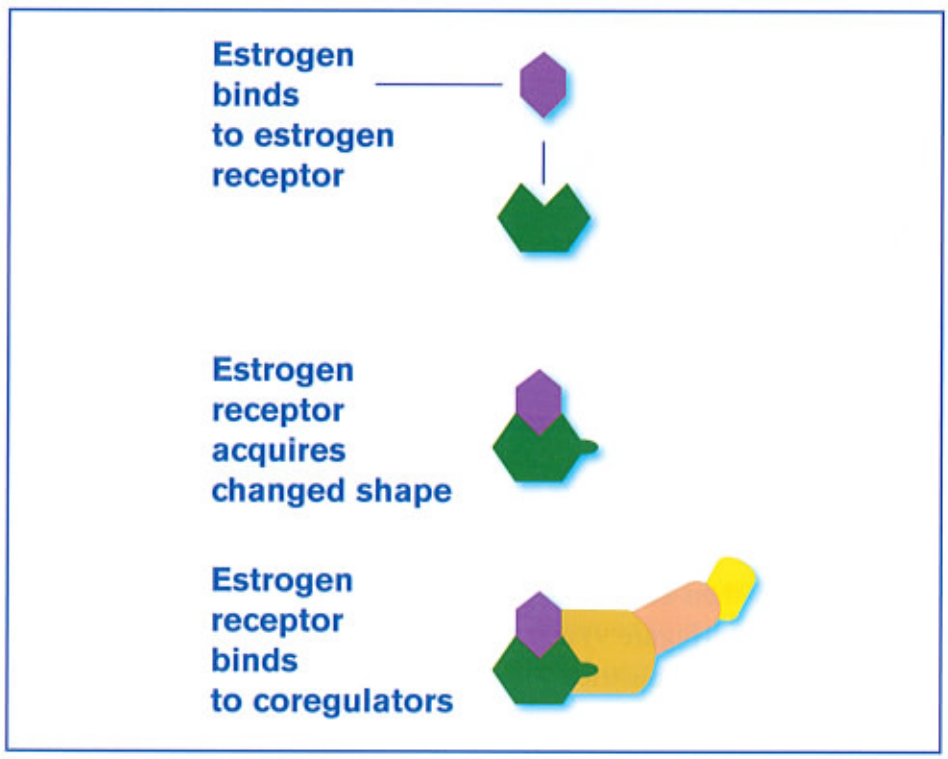


Figure 8: Estrogen Receptor Changes Shape to Bind Coregulators - When estrogen binds to the estrogen receptor (ER), it causes the ER to change its shape slightly. The change in shape allows coregulators to also bind to the ER. Coregulators are proteins in the cell that attach to the estrogen-ER complex. Depending on which coregulators bind to the ER, they may inhibit or enhance the estrogen stimulated transcription or translation of the cell's DNA into proteins. **Please note:** For illustrative purposes, dimerization (pairing) of the estrogen receptor, as described in the text, is not shown. *Designed by Corporate Press, adapted from the National Cancer Institute, National Institutes of Health.*

Since estrogen is essential for fertility, scientists looking for new contraceptives wanted to find drugs that could block the action of estrogen by blocking the ER. Chemists began synthesizing compounds that were shaped like estrogen, but didn't act like estrogen. The first of these "anti-estrogens" was MER-25. Elwood

Figure 10: The 'Father of Tamoxifen' -Widely hailed as the "father of tamoxifen," Dr. V. Craig Jordan, pictured here (second from left) with his 1999 laboratory group, is credited with many of the pivotal discoveries leading to our current understanding of how tamoxifen and related drugs work. Beginning with his doctoral studies, Jordan reinvented tamoxifen from a failed contraceptive and became a driving force behind its use as a treatment and preventive. *Photo courtesy of Dr. V. Craig Jordan.*

Jensen showed in 1962 that MER-25 blocks the binding of estrogen in the rat uterus. Unfortunately, MER-25 was too toxic for use in humans. Another of the "anti-estrogens" studied as a potential contraceptive, clomiphene, turned out to have the opposite effect in women. It is used today as a fertility drug.

In the 1960s, Arthur Walpole and Michael Harper were studying potential contraceptive drugs synthesized by their colleague, Dora Richardson (Figure 9) at Imperial Chemical Industries (ICI) Pharmaceuticals in England. One of the compounds, ICI 46,474 (now called tamoxifen), was, like clomiphene, a bust as a contraceptive. In 1967, Harper and Walpole noted the paradox that tamoxifen blocked estrogen in some tissues and animals, but mimicked the action of estrogen in others. That astute observation would be the key to the eventual success of drugs like tamoxifen…but not yet.

Walpole had an interest in cancer biology and knew of the link between estrogen and breast cancer. He suspected that tamoxifen might be developed as a cancer drug. ICI was not interested in the project, so Walpole encouraged other scientists to pursue this avenue of research. In 1967, V. Craig Jordan (Figure 10) was a student intern at ICI, where he met Walpole. Jordan took up the tamoxifen project for his doctoral research. Jordan's work with rats showed that tamoxifen could block estrogen from binding to the ER, shrink existing tumors, and prevent chemically induced mammary tumors. Although he was sometimes met with resistance from the scientific community, Jordan continued to study tamoxifen for decades.

Armed with data from the laboratory, investigators began clinical studies with tamoxifen, with encouraging results. Tamoxifen could temporarily reduce tumors in some patients with late-stage, widely disseminated breast cancer. The side effects of tamoxifen were much milder that those produced by conventional chemotherapy. The drug was approved as a treatment for advanced breast cancer in the UK in 1973 and in the US in 1978.

What about women in whom cancer was treated early? Tumors can be removed surgically, but there is always a chance that some cancer cells have escaped and spread to other parts of the

body. These so-called micrometastases could then cause the cancer to recur after the tumor is removed. Again, work in the laboratory could provide clues to tamoxifen's use in humans. Animal studies had indicated that long-term treatment with tamoxifen could prolong survival when used in the early stages of the disease, so tamoxifen was studied in women with early cancer. After surgical removal of a tumor, tamoxifen would theoretically target micrometastases and prevent recurrences. Clinical studies showed that a five-year course of tamoxifen was indeed effective at preventing recurrence of tumors. This benefit was only seen in patients whose original tumors were ER-positive. Remarkably, the benefits of five years of tamoxifen continue, even ten years after treatment is completed.

As Jensen had proposed, the presence of the ER can predict if a tumor is likely to be sensitive to tamoxifen. Today, it is routine practice to test breast tumors for the presence of the ER. Women with ER-positive tumors are offered tamoxifen treatment, which is effective in 50–60% of these cases. Women with ER-negative tumors are offered conventional chemotherapy, since tamoxifen is effective in less than 10% of these cases.

More laboratory studies suggested a further use for tamoxifen—in cancer prevention. Tamoxifen given to laboratory animals can reduce the number of animals that eventually develop cancer. In the 1980s, a study of 13,800 pre- and post-menopausal women at high risk for breast cancer showed that tamoxifen can cut the risk of developing breast cancer in half. Tamoxifen was approved for the reduction of breast cancer risk in 1998.

Another Drug, Another Application

One problem associated with tamoxifen was resistance. As mentioned previously, only 50–60% of women with ER-positive tumors respond to tamoxifen. Some ER-positive tumor cells are not inhibited by tamoxifen, and some are sensitive at first, but later become resistant. As scientists looked for a way to treat tamoxifen-resistant cancers, they developed a similar drug, then called keoxifene. Unfortunately, keoxifene didn't work well in the laboratory or in women with tamoxifen-resistant breast cancers, so clinical research on the drug stopped. (It turns out that resistance to tamoxifen and keoxifene are caused by the same cellular change.) Jordan, however, continued to study keoxifene in the laboratory.

Another troubling problem with tamoxifen was that women taking the drug (especially post-menopausal women) were two to four times more likely to develop cancer of the lining of the uterus (the endometrium). This was puzzling, since, if tamoxifen was blocking the action of estrogen, it should have a protective effect on uterine cancer just as it did for breast cancer. Instead, it did the opposite, and increased the risk of uterine cancer.

Scientists were also concerned about another possible side effect of tamoxifen. Since estrogen is important for maintaining bone density and strength, they feared that tamoxifen, by blocking the action of estrogen in the bones, might worsen osteoporosis and put women at risk for broken bones.

Surprisingly, just the opposite happened. In a study published in *The New England Journal of Medicine* in 1992, Richard Love and his coworkers at the University of Wisconsin-Madison reported that postmenopausal women being treated with tamoxifen actually had enhanced bone density. What had been a concern as a side effect had become an added benefit.

Because of Love's study and Jordan's ongoing research, keoxifene, the failed anti-estrogen, was taken out of retirement and tested as a drug to enhance bone density. Keoxifene was effective as a preventative treatment for osteoporosis and it was approved for that use in 1997 under the name of raloxifene. (See previously mentioned *Breakthroughs in Bioscience* article on osteoporosis.)

More recently, a study of more than 19,000 high-risk post-menopausal women compared the effectiveness of raloxifene

and tamoxifen. Preliminary results released in April of 2006 showed that raloxifene was as effective as tamoxifen at preventing invasive breast cancer. Both drugs cut the risk in half. Interestingly, tamoxifen but not raloxifene reduced the risk of non-invasive cancers, called ductal carcinoma in situ and lobular carcinoma in situ (DCIS and LCIS). The two drugs were also equally effective at reducing bone fractures. Raloxifene, however, was less likely than tamoxifen to cause uterine cancers or blood clots. Although raloxifene is ineffective against tamoxifen-resistant tumors, continued study will determine if it will be a useful alternative to tamoxifen.

A Most Intriguing Paradox

Normally, estrogen has different effects on different tissues, some good and some bad. In bone, for example, estrogen has a positive effect, promoting the deposition of calcium. In the breast, however, estrogen enhances the growth of tumor cells, a negative effect.

Tamoxifen can also have opposing effects in different tissues. It acts as an anti-estrogen in the breast and may also produce hot flashes in some women, suggesting that it is acting as an anti-estrogen in the brain. In other tissues, however, it acts like estrogen, with positive or negative effects. In the uterus, it increases risk for cancer. In the liver it increases the risk of blood clots (a bad thing), but also decreases blood levels of the "bad cholesterol" LDL (a good thing). In the bones, tamoxifen appears to have estrogen-like activity and enhances bone density in postmenopausal women, but in premenopausal women, bone density appears to be decreased by tamoxifen. The action of tamoxifen, and whether it has a positive or negative effect, depends on the type of tissue that it is interacting with, as well as the hormonal environment in which it is operating.

If tamoxifen were acting as a pure anti-estrogen, it would produce the opposite effects of estrogen in every tissue. It would inhibit the growth of both breast and uterine cancer and reduce bone density. Instead, it increases the risk of uterine cancer and enhances bone density. In the uterus and bones it acts like estrogen, not like an anti-estrogen. The same drug, in different tissues, can mimic estrogen or act in the opposite way.

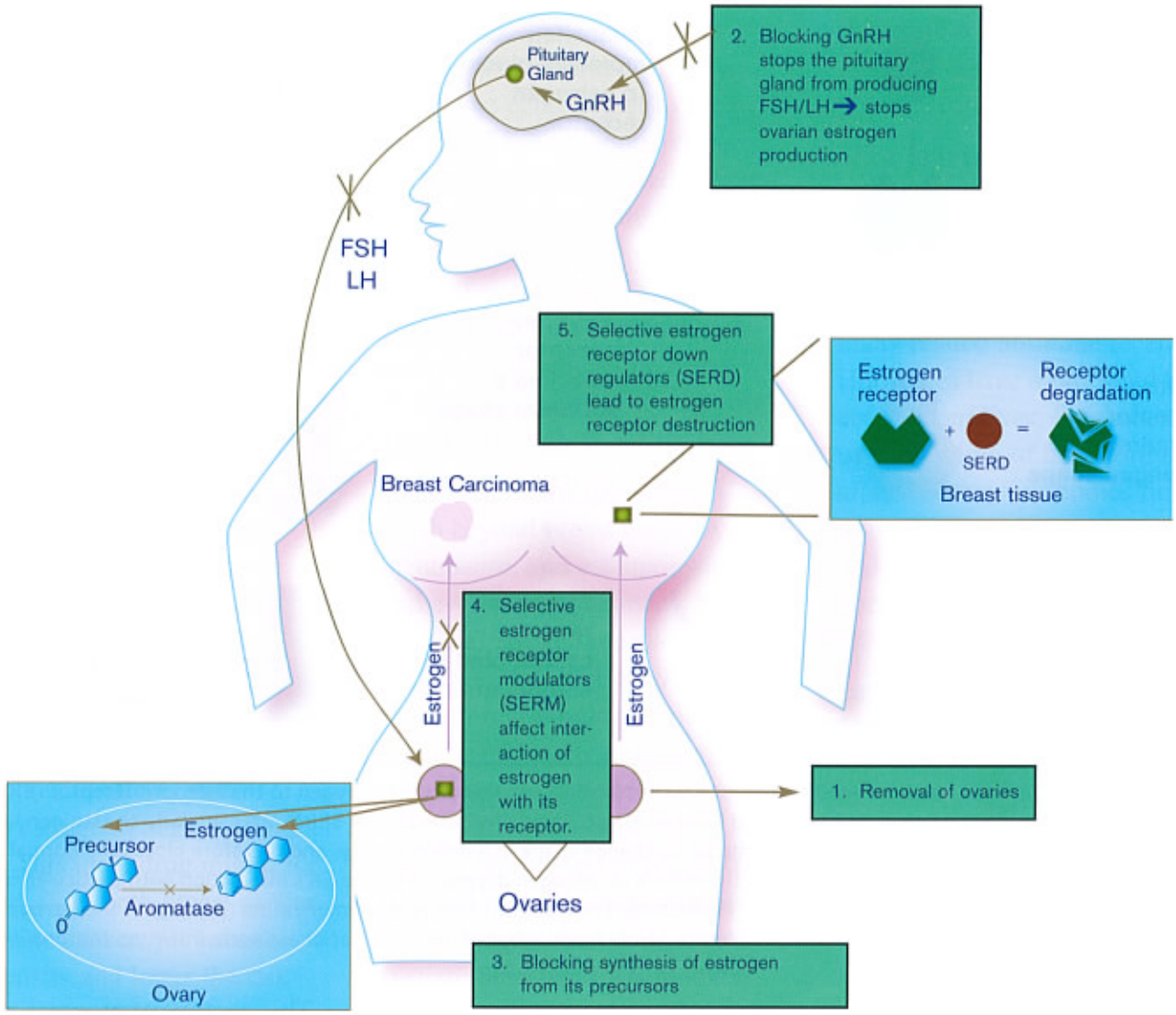
Clearly, "anti-estrogen" was not an accurate description of tamoxifen and raloxifene. A new term was needed: selective estrogen receptor modulator (SERM).

What's a SERM?

The success of tamoxifen has been remarkable. It has prolonged the lives of millions of women, but its discovery was serendipitous. When it was introduced, very little was known about how drugs like tamoxifen worked. Originally, it was thought that these drugs simply blocked the ER, preventing estrogen from binding and inducing transcription. Further analysis showed that SERM action is not that simple and is, like the action of estrogen, dependent on changes in the shape of the receptor.

In 1992, Bert O'Malley and his coworkers proposed that when a SERM binds to a hormone receptor, it changes the shape of the receptor so that it cannot promote transcription. Further studies by other groups showed that, while estrogen and SERMs change the shape of the ER, the changes produced by estrogen and SERMs are different. What's more, different SERMs produce different structural changes. Since the shape of the receptor is so important to how it interacts with the other molecules in the cell, estrogen, tamoxifen, and raloxifene might cause the receptor to interact with different molecules, producing different effects. (Figure 11)

But why does the same SERM produce different effects (estrogen-like or anti-estrogen) in different cells? The answer appears to be in O'Malley's "magical" coregulators, the proteins that interact with the activated estrogen (or SERM)-bound ER. The shape of the activated receptor determines which coregulators will bind to it. Different coregulators appear to bind to different parts of the receptor. The effect of a SERM on the cell depends on the cumulative interactions of all the coregulators with which it interacts. Each dif-



Sidebar 2: Lacassagne's prediction revisited.

In 1936, Antoine Lacassagne made this startlingly accurate prediction: "…one is led to imagine a therapeutic preventative for subjects predisposed by their heredity to this cancer. It would consist-perhaps in the very near future when the knowledge and use of hormones will be better understood-in the suitable use of [an antagonistic] hormone…"

There are at least five ways Lacassagne's proposal to block the action of estrogen might be accomplished. George Beatson hit on the first one when he removed the ovaries of women with advanced breast cancer. (1) By removing the ovaries, or other organs that induce the ovaries to make estrogen, one can prevent estrogen from being produced by the ovaries. This can be accomplished surgically, as Beatson did, or by using radiation to stop the ovaries from producing estrogen. In the 1950s and 1960s, removal of the adrenal glands (a source of estrogen precursors, especially in postmenopausal women) was effective in about a third of cases.

Instead of removing or inactivating an estrogen-producing organ, one could (2) block messengers that induce ovaries to make estrogen. The removal of the pituitary gland (hypophysectomy) was also used in the 1950s and 1960s, since the pituitary gland produces hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which together induce the ovaries to secrete estrogen. More recently introduced drugs called GnRH (gonadotropin releasing hormone) antagonists block the signal (GnRH) with which the brain tells the pituitary gland to release LH and FSH.

A third way (3) would be to block the synthesis of estrogen from its precursors. The enzyme that controls the last step of estrogen synthesis is called aromatase. Aromatase inhibitors prevent that last step from occurring in the ovaries, breasts, or other tissues. Aromatase inhibitors are safe and effective in postmenopausal women.

Selective estrogen receptor modulators (SERMs) were originally developed to (4) block the interaction of estrogen with its receptor. Their action is, in fact, more complicated than that, and is described in more detail in the text.

Finally, the ER can be removed (5) by drugs called selective estrogen receptor down-regulators (SERDs). When these drugs bind to the ER, they destabilize it and tag it for destruction by the cell's "garbage disposal" system.

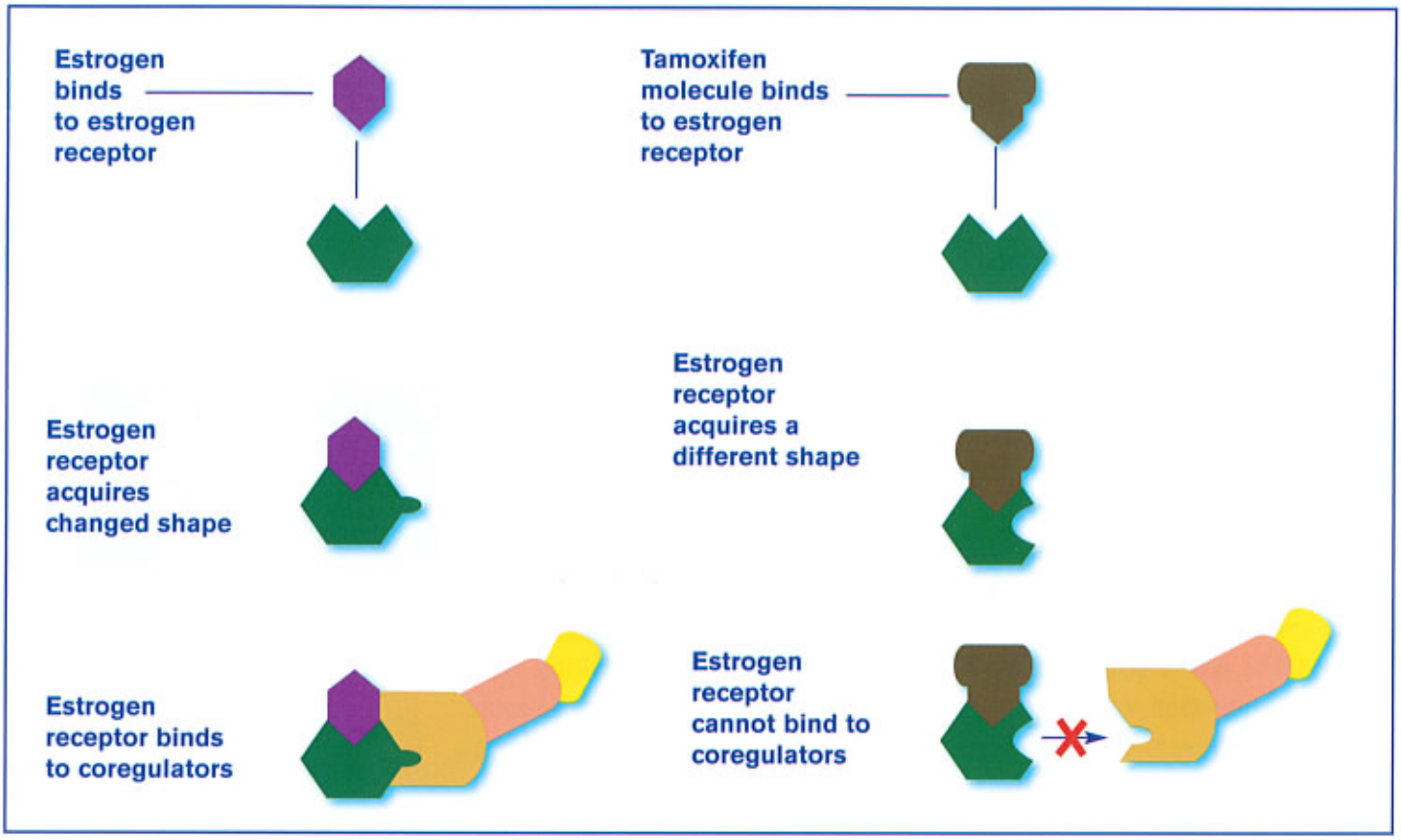


Figure 11: Selective Estrogen Receptor Modulators (SERMs)—Normally, the binding of estrogen to the estrogen receptor (ER) causes the ER to change its shape, bind to coregulators and have an effect. Tamoxifen, one kind of SERM, acts similarly to estrogen in that it, too, binds to the ER. However, tamoxifen causes the ER to change shape in a different way, and therefore blocks the normal binding of coregulators associated with estrogen. Because the effects of estrogen depend upon the ER's interaction with particular coregulators, tamoxifen effectively inhibits estrogen action. **Please note:** For illustrative purposes, dimerization (pairing) of the estrogen receptor, as described in the text, is not shown. *Designed by Corporate Press, adapted from the National Cancer Institute, National Institutes of Health.*

ferent kind of cell, it seems, possesses a different complement of the more than fifty proteins that have been shown to interact with the receptor. The unique effect of each SERM on each target cell is due to the combined effect of how the SERM changes the shape of the receptor and how the coregulators present in a particular cell interact with the altered receptor. In some cells, the SERM (i.e.-tamoxifen) will act like estrogen. In other cells it will not.

The Shape of Drugs to Come

More than a century has passed since George Beatson treated breast cancer patients by removing their ovaries. The introduction of tamoxifen as a cancer treatment was a high point, but not the only one. More recent work has resulted in new drugs, and in new ways to use them. Today breast tumors are routinely tested for the presence of the ER, and cancer treatment is tailored to the characteristics of the tumor. SERMs are now important tools in the treatment and prevention of both breast cancer and osteoporosis.

This century of progress was not due solely to the work of the few investigators mentioned here. It required hundreds of scientists in fields from physics to biochemistry to molecular genetics. It required the development of new techniques and equipment and the participation and cooperation of university, industry, and government labs. Basic research in seemingly unrelated fields converged in tamoxifen, and tamoxifen was just the beginning.

With the understanding that the effects of estrogen or SERMs depend on how they cause the ER to interact with coregulators within each cell, researchers are faced

with the (admittedly daunting) task of determining which coregulators need to interact with the receptor to produce the desired effect (either estrogen-like or anti-estrogen) in each cell. This information can be used to develop new SERMs or even drugs that directly target coregulators.

Today, the goal in SERM development is rational design, that is, to design a drug using knowledge about how drugs change the shape of the ER and then change how it interacts with coregulators. This is a more productive approach than the somewhat random process used to develop tamoxifen. It now seems possible to design the perfect SERM—a drug that both mimics estrogen's positive health effects and inhibits estrogen's negative health effects. This ideal drug might, for example, fight both breast cancer and uterine cancer, yet enhance bone density and lower the risk of heart disease.

Next-generation SERMs, such as bazedoxifene (currently in clinical trials), hold promise as drugs that come closer to the perfect SERM. New approaches, such as combining bazedoxifene with a mixture of estrogens, are also being tested. By combining the best properties of estrogen and SERMs, this approach may help prevent cancer and osteoporosis, while easing menopausal symptoms, such as hot flashes.

The benefits of ER research reach beyond breast cancer, menopause, and osteoporosis, and even beyond women's health. Another estrogen receptor, called ERβ, was reported in 1996. ERβ has different binding properties than the originally described receptor (now called ERα). While ERα is found in the breasts, uterus, and bones, ERβ is found in the ovaries, prostate, testes, lungs, brain, and immune system. Selective ERβ modulators are being tested for a wide variety of conditions, including rheumatoid arthritis, inflammatory bowel disease, and even prostate cancer.

Just as researchers in ER biology have benefited from other fields of research, ER research has wide-reaching potential benefits, notably in the study of the many related hormone receptors. Hormones have a profound influence on our lives. Fundamental understanding of how hormones affect the way our bodies function will translate into better understanding of these functions, opening doors to new ways to improve the quality of our lives.

Biographies

Jacqueline Jaeger Houtman, Ph.D. writes about biomedical science from Madison, Wisconsin. She enjoys writing for physicians, scientists, middle school students, and the general public. She has written for The Dana Foundation, World Book, and scientific journals, including *Clinical and Experimental Allergy* and *Journal of NeuroVirology*. She can be reached at jjhoutman@nasw.org.

Donald P. McDonnell, Ph.D. is a professor of pharmacology and cancer biology and Glaxo-Wellcome Professor of molecular cancer biology at the Duke University Medical Center, where his research focuses on the role of estrogen and progesterone in the pathology of hormone dependent breast cancers. McDonnell's lab aims to facilitate the development of novel therapeutically important molecules for the treatment of breast cancer and other important endocrinopathies. He is widely published, and is a leading national spokesman on issues related to estrogen, breast cancer, and research into human diseases. McDonnell's work has been recognized through awards by the scientific community, including the Richard E. Weitzman Memorial Award and Ernst Oppenheimer Award.

Suggested Reading

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Also

The American Cancer Society www.cancer.org

The Susan G. Komen Breast Cancer Foundation www.komen.org

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